

hydrolysed,  $n/2$  molecules of the corresponding biose is obtained. If now the biose is hydrolysed, the net result is a total hydrolysis of the polysaccharide, and it is evident that the mean value of  $[\text{Sp}]_D$  is the mean of  $[\text{Sp}]_D$  for the central bond and that for the biose. For starch the  $[\text{Sp}]_D$  for central bonds is thus calculated as  $184^\circ$ , in good agreement with the value found by Blom and Schwartz. In the series cellobiose to celloheptaose<sup>2</sup> and xylobiose to xylohexaose<sup>3</sup> there are also great differences between the rotation contributions of central and terminal bonds. The plot of  $[\text{M}]_n/n$  against  $n-1/n$ , where  $[\text{M}]_n$  is the molecular rotation and  $n$  the degree of polymerization, yields a straight line, however, which according to Freudenberg<sup>4</sup> means that the linkages in the series are uniform. In the maltose series only the next member, maltotriose, is known<sup>5</sup>, but from the molecular rotation of this substance ( $+807^\circ$ ), of maltose ( $+470^\circ$ ), and of glucose ( $+95^\circ$ ), the  $[\text{Sp}]_D$  for a central bond is calculated as  $204^\circ$ .

Thus Blom and Schwartz have merely confirmed experimentally the expected value of  $[\text{Sp}]_D$  for central bonds in the starch molecule. They have not adduced any evidence supporting the occurrence of furanosidic bonds in starch. If the mere fact that the  $[\text{Sp}]_D$  of a central bond is different from that of a terminal bond should constitute an argument for this assumption, it could equally well be applied to all tri- and higher saccharides.

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## Crystalline Rhodanese

BO H. SORBO

*Biokemiska Avdelningen, Medicinska Nobel-institutet, Stockholm, Sweden*

Recently we reported a method for the partial purification of rhodanese<sup>1</sup> from beef liver. The enzyme has now been further purified and obtained in the crystalline state.

The method of purification was as follows: Beef liver was extracted and the extract fractionated with ammonium sulfate at pH 3.8 and 7.8 as described before<sup>1</sup>. The enzyme was then dialyzed against 0.01 *M* sodium acetate and the pH of the dialyzed solution adjusted to 4.9. Fractionation with acetone was then carried out at  $-5^\circ\text{C}$  and the precipitate appearing between 30 and 50 % by volume acetone was collected and dissolved in cold 0.01 *M* sodium acetate. The remaining acetone was removed by dialysis against 0.01 *M* sodium acetate. The pH of the solution was then adjusted to 4.5, the enzyme precipitated with ammonium sulfate at 40 % saturation and the precipitate dissolved in 0.01 *M* sodium acetate. When a 0.5 % solution of this preparation was examined in the "Spinco" ultracentrifuge of this department, only one homogeneous boundary with  $S_{20} = 2.76 \cdot S$  was observed. The solubility of a similar preparation in ammonium sulfate of varying concentration was then studied at room temperature and pH 5. The logarithm of solubility for the enzyme was found to be proportional to the concentration of ammonium sulfate. An amorphous precipitate was obtained, but when those supernatants, which still contained enzymatic activity were brought to  $+2^\circ\text{C}$ , a pronounced prethixotropy slowly developed. No crystals were, however, visible in the microscope, but later experiments showed, that rhodanese could be crystallized from ammonium sulfate at

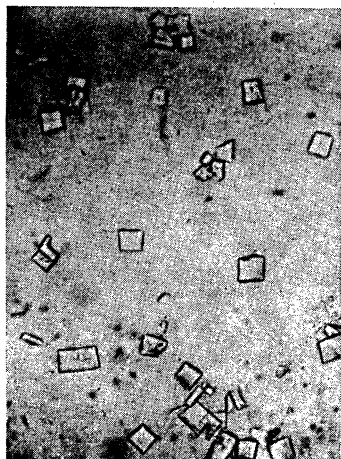


Fig. 1. Crystalline rhodanese from ammonium sulfate, pH 7.8.  $\times 308$ .

pH 7.8 as small, strongly lightdispersing cubes or larger rectangular plates, see Fig. 1. The specific activity of the preparation was raised from 0.84 to 1.0 by crystallisation, the crystalline enzyme being purified 650-fold from the starting material.

1  $\mu\text{g}$  of crystalline rhodanese was able to form 2.84  $\mu$ -equivalents of thiocyanate during 5 minutes incubation at 20° C. Assuming the molecular weight of the enzyme = 35 000 this corresponds to a turnover number of 20 000  $\text{min}^{-1}$ . Our test system was of pH 8.75 and contained in a final volume of 2.5 ml 0.1 *M* thio-sulfate, 0.05 *M* cyanide, 0.08 *M* phosphate and 0.125 mg bovine albumin. The albumin was found to be necessary in order to obtain full activity with the more purified enzyme preparations.

The crystalline enzyme gave a colorless solution with an ordinary protein absorption spectrum, which was not changed by the presence of 0.05 *M* thiosulfate at pH 7.4. No formation of an enzyme-substrate compound could thus be observed spectrophotometrically.

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A detailed report will be published in a near future.

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## On the Principle of Thermal Interaction

JAN KROGH-MOE

*Institutt for Teoretisk Kjemi, Norges Tekniske Høgskole, Trondheim, Norway*

In a recent work Holtan<sup>1</sup> states a principle of thermal interaction: "In a non-isothermal process there is an interaction between the flow of heat and the transport phenomena in such a way that the loss of work (loss of potential energy) for the transport of entropy bound to chemical substance can be compensated partly or completely by the transport of free entropy contained in the flow of heat in the opposite direction."

This principle is embodied in the principle of least dissipation of energy. It is thus possible to prove the principle of thermal interaction for the cases where the principle of least dissipation of energy is valid. The restrictions and variations with respect to which the dissipation is a minimum, are stated by Wergeland<sup>2</sup>.

For a process under consideration we can split the total rate of increase  $\dot{S}$  in the entropy of the system in two parts:

$$\dot{S} = 2\Phi - \dot{S}^*$$

In the stationary state  $\dot{S}$  will be zero, and the dissipation function  $\Phi$  can be shown to be a minimum. Consequently the total flow of entropy across the surface of the system,  $\dot{S}^*$ , will be a minimum.